A. Animal Pharmacokinetic and Biodistribution Studies

Our results are described in the attached preprints (Fujita et al 2000) (Zoghbi et al 2001) and briefly reviewed below.

The *in vivo* properties of a new radioiodinated probe of the α4β2 nAChR, [I-123]5-I-A-85380 were evaluated baboons. The labeled product was prepared in a yield of 48.4 ±17.5% (mean ± SEM; n = 16) by iododestannylation of the t-BOC protected trialkylstannyl precursor 5-trimethylstannyl-3-[1-*tert*-butoxycarbonyl-2(S)-azetidinylmethoxy]pyridine in the presence of Chloramine-T (Musachio et al 1999) followed by high pressure liquid chromatography (HPLC) purification to give a product with radiochemical purity of 97.4 ± 1.5% and specific activity > 5,000 Ci/mmol.

After injection of 0.22 ± 0.12 mCi/kg [I-123]5-I-A-85380 (15.4 ± 8.4 mCi/70 kg), heart rate (-2.1 ± 5.0 /min) or body temperature (-0.2 ± 0.3 °C) did not show a significant change within an hour. High resolution SPECT imaging demonstrated high tracer uptake in the thalamus, medium uptake in the striatum and cerebral cortices and low uptake in the cerebellum. Plasma metabolites and pharmacokinetics were analyzed in baboons by acetonitrile denaturation of plasma proteins followed by HPLC analysis. This tracer produced two metabolites, one polar and the other lipophilic. Under equilibrium conditions achieved by bolus plus constant infusion, mean fractions in plasma activities were parent: 26.4%, lipophilic metabolite: 15.7%, and polar metabolite: 58.0%. The plasma protein bound fraction, determined by ultrafiltration, was, 38.8 ± 1.0%. The arterial input function was characterized by the sum of three exponential terms with mean half-lives of 0.79, 27.5, and 98.6 min, respectively.

Quantitation of the tracer uptake was performed by applying a pair of bolus/kinetic and bolus plus constant infusion (B/I)/equilibrium studies in each of three baboons. Bolus studies were performed by intravenous injection of 191 – 226 MBq [I-123]5-I-A-85380 and image acquisition for 289 – 367 min. The data were analyzed with one- and two-tissue compartment models. B/I studies were performed by a bolus injection (74 – 132 MBq) followed by 468 – 495 min infusion with B/I ratio 4.8 – 5.0 h. The distribution volumes in the thalamus were measured in these two paradigms. To study if the cerebellum was appropriate as a receptor-poor region, displacement studies were done in two baboons using B/I paradigm with s.c. injection of (-)-cytisine (0.8 and 1.0 mg/kg). The kinetics of this tracer was best described by a one-tissue compartment model. The two-compartment model provided poor estimation of rate constants. The total (specific plus nondisplaceable compartments) distribution volumes (V_T') agreed between bolus (28.2 mL/mL) and B/I paradigms (34.5 mL/mL) (average % difference in V_T': 16.8%). (-)-Cytisine (0.8 and 1.0 mg/kg) displaced 70 and 72% of the radioactivity in the thalamus and 36 and 55% in the cerebellum, indicating that the latter was not appropriate as a receptor-poor region. These results demonstrated the feasibility of quantifying α4β2 nAChRs using [I-123]5-I-A-85380 and support the use of V_T' as an appropriate outcome measure.

Radiation absorbed dose was estimated based on MIRDOSE 3 by DB Vaupel, PhD at NIDA using CD-1 mice (n = 6, 3 males and 3 females). Estimates of radiation absorbed dose gave 0.268, 0.24, 0.14, and 0.0725 rad/mCi to the urinary bladder wall, thyroid, lower large intestine wall, and ovaries, respectively. Estimated radiation absorbed doses in all organs are shown in the table below.